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Basic Mechanisms in Allergic Disease

Immune-microbiota interaction in Finnish and Russian Karelia young people with high and low allergy prevalence

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Abstract

Background: After the Second World War, the population living in the Karelian region was strictly divided by the “iron curtain” between Finland and Russia. This resulted in different lifestyle, standard of living, and exposure to the environment. Allergic manifestations and sensitization to common allergens have been much more common on the Finnish compared to the Russian side.

Objective: The remarkable allergy disparity in the Finnish and Russian Karelia calls for immunological explanations.

Methods: Young people, aged 15–20 years, in the Finnish ($n = 69$) and Russian ($n = 75$) Karelia were studied. The impact of genetic variation on the phenotype was studied by a genome-wide association analysis. Differences in gene expression (transcriptome) were explored from the blood mononuclear cells (PBMC) and related to skin and nasal epithelium microbiota and sensitization.

Results: The genotype differences between the Finnish and Russian populations did not explain the allergy gap. The network of gene expression and skin and nasal

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microbiota was richer and more diverse in the Russian subjects. When the function of 261 differentially expressed genes was explored, innate immunity pathways were suppressed among Russians compared to Finns. Differences in the gene expression paralleled the microbiota disparity. High *Acinetobacter* abundance in Russians correlated with suppression of innate immune response. High-total IgE was associated with enhanced anti-viral response in the Finnish but not in the Russian subjects.

Conclusions and clinical relevance: Young populations living in the Finnish and Russian Karelia show marked differences in genome-wide gene expression and host contrasting skin and nasal epithelium microbiota. The rich gene-microbe network in Russians seems to result in a better-balanced innate immunity and associates with low allergy prevalence.

KEYWORDS

environment and hygiene hypothesis, epidemiology, omics- and systems biology

1 | INTRODUCTION

There is need for understanding why many chronic inflammatory conditions, allergy and asthma among them, are increasing in the rapidly urbanizing world. We have previously reported a stark allergy contrast between the Finnish and adjacent Russian Karelian populations.¹ These areas are socio-economically distinct but geo-climatically similar, and the populations partly share the same ancestry.² Allergy and asthma have been systematically more common on the Finnish side. For example, in the early 2000s, hay fever in schoolchildren was almost non-existent in Russian Karelia. Only 2% were sensitized to birch pollen, compared with the prevalence of 27% in the Finnish Karelia. Seropositivity to some pathogens, microbial content in house dust and drinking water were associated with low allergy prevalence in Russia.³ The same schoolchildren have been followed to young adulthood, and the allergic conditions remained 3- to 10-fold more common in Finland.⁴ Interestingly, both skin and nasal microbiota were also strongly contrasting between the populations, the microbiota being more diverse among Russian subjects.

In the recent meta-analysis of genome-wide asthma liability genes, nine new loci were added to the 21 previously identified.^{5,6} The findings confirmed that immunologically related mechanisms are key players in asthma risk. Asthma-associated genes were expressed in both immune and epithelial cells modulating airway inflammation, especially to viruses and bacteria. The prevalence of physician-diagnosed asthma in Finland is close to 10%,⁷ and the recognized genetic liability might play a role in at most one third of patients. Environmental and lifestyle changes seem to determine substantial proportion of immune tolerance variation between individuals and populations.^{4,8-11} They modify the richness, diversity, and composition of the human microbiome and immune regulation.¹²

We hypothesized that the allergy gap between Finnish and Russian Karelia is largely attributed to differences in lifestyle and living environment, affecting exposure to environmental microbiota. We explored differences in their genetic variation, gene expression (transcriptome) profiles and association with commensal microbiota. Genuine inflammatory pathways were searched to explain the variation of immune regulation between the two populations.

2 | METHODS

2.1 | Study subjects and design

The study subjects have been previously recruited for a long-term study and provided written informed consent, and the protocol has been approved by an institutional ethics committee (202/E7/2003; TY2012202/53/2012; TY2012202/25/2014).¹ The original clinical data have been presented elsewhere.⁴ For the present analyses, blood samples and skin microbiota results from the Finnish ($n = 69$:30 males, 39 females) and Russian ($n = 75$:36 males, 39 females) young people, aged 15-20 years, were available. Selection of study subjects and clinical characteristics are described in more detail in the Appendix S1.

2.2 | Total and allergen-specific serum IgE (sIgE)

Total and allergen-specific sIgE measurements against eight common inhalant allergens (timothy grass, birch, mugwort, *Cladosporium herbarum*, horse, cat, dog, and house dust mite, that is *Dermatophagoides pteronyssinus*) and six common food allergens (cow's milk, hen's egg, cod fish, soy, wheat, and peanut), and Phadiatop® for inhalant and food allergens are shown in Table S1 in Appendix S1.

2.3 | Genome-wide association analysis

In order to ascertain eventual differences in the genetic composition of the two population used in this study, we analyzed genotype data of 122 samples (60 FIN and 62 RUS) from our cohort. For each individual, a panel of 629324 SNPs has been genotyped using Illumina GlobalScreeningArray-24v2-0_A1 beadchip platform.

Population stratification has been investigated using PLINK and fastStructure tools. “*Identity by State*” (IBS) and “*Identity by Descend*” measures were computed for the given populations, and “*Permutation test for between group IBS differences*” (from PLINK suite) has been used to test the association between genetic distance among samples and the phenotype of interest. Identification of SNPs associated with nationality has been performed using “*Basic case/control association test*” with multiple test correction from PLINK suite. Finally, principal component analysis PCA was performed on sample genotypes using SNPRelate R package. For more information about the analysis, see Appendix S1.

2.4 | Analysis of the skin and nasal microbiota

The processing of microbial samples obtained from skin and nasal epithelium, DNA extraction, sequencing, and sequence processing is fully described elsewhere.⁴ For practical reasons, it was not possible to receive and transport fecal samples, reflecting gut microbiome, from the teenagers. From the samples analysed in,⁴ we only used those with a corresponding PBMC transcriptome sample available.

2.5 | Immunological analysis

Mononuclear cells (PBMC) were separated from whole blood (BD Biosciences Pharmingen) and frozen until analysed. The thawed PBMCs were left unstimulated or stimulated with the heat-inactivated *Acinetobacter lwoffii* at 10 µg/mL for 6–24 hours in complete RPMI-1640 medium at 37°C and 5% CO₂, as previously described.¹³

2.6 | Gene expression

2.6.1 | RNA isolation

Total RNA was extracted from the blood leucocytes (Allprep DNA/RNA Universal Kit, Qiagen). The yield and purity of RNA was measured (Nanodrop spectrophotometer, Qubit fluorometer), and RNA integrity was quantified (Agilent RNA Pico kit).

2.6.2 | Microarray analysis

A total of 100 ng of total RNA was amplified, labelled with Cy3 and Cy5 dyes, and hybridized to human microarrays (Agilent protocols,

SurePrint G3). Raw data were monitored for quality, and quantile normalized (Bioconductor package Limma).¹⁴ Batch effects originating from labelling and array variance were removed (SVA package with Combat function).¹⁵ For identification of differentially expressed genes, a linear model was fitted to the data (using gender, dye, and array as covariates), and pairwise comparisons were done using the empirical Bayes method. Transcriptomes were defined based on a fold change of 1.5 or greater and a Benjamini-Hochberg adjusted p-value less than 0.05. For more information about cut-off levels for differentially expressed genes, see Figure S3 in Appendix S1. Functional enrichment analyses were performed using web-based tools (<http://amp.pharm.mssm.edu/Enrichr/>) and the Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity). For information about the gene network inference and response module analysis, see Appendix S1.

2.6.3 | Real-time quantitative PCR

mRNA levels of IL-6 and IL-1beta were analysed (quantitative RT-PCR, TaqMan chemistry, 7500 Fast Real-Time PCR System, Applied Biosystems, Life Technologies). Polymerase chain reaction amplification of the endogenous 18S rRNA and GAPDH was used for each sample to control sample loading and to allow normalization between samples. The results are expressed as relative units (RU), which were calculated by the comparative C(T) method according to the manufacturer's instructions.¹⁶

2.7 | Numerical analysis

2.7.1 | Multivariate analysis

Firstly, redundancy analysis (RDA) was used to model the dispersion of either genes or skin microbes (Operational Taxonomic Units, OTUs), as a function of national identity (Finnish/Russian), also correcting for subject age and gender. Here, the first constrained RDA axis presents data variation that can be attributed to between-population differences: for microbes the 1st axis accounted for 8.8% of all variation, and for genes, the 1st axis accounted for 55% of all variation. Plotting the two RDA axes against each other allows to illustrate between-population differences in microbial composition and gene expression. This analysis was performed with the rda function in R (vegan package¹⁷) for genes and dbrda function for microbes, assuming Bray-Curtis dissimilarity between samples.

Next, we tested whether samples with similar gene expression also tend to have similar microbial composition. This was done using Mantel correlation. To this end, we calculated between-sample Euclidean distance in gene expression space and between-sample Bray-Curtis dissimilarity in microbial composition (both skin and nasal), for the full data as well as within populations. Statistical significance was assessed

using 999 random permutations. This analysis was performed with the mantel function in R (vegan package¹⁷). Mantel correlation coefficients and associated permutation *P*-values are given in Appendix S1.

2.7.2 | Network inference

Co-expression networks were generated from combined data of genes and skin and nasal OTUs (bacteria and fungi) for both populations. Prior to analysis, OTUs within each sample type were trimmed, so that microbes with prevalence less than 70% were excluded. This was done to reduce the number of features, but also to ensure that the associations between the remaining features can be estimated meaningfully. This filtering resulted in 167 microbes remaining, 109 of which were shared between populations, whereas 58 microbes were unique to Russian subjects. For detailed description of the network inference procedure, see Appendix S1.

3 | RESULTS

3.1 | Genetic variability between the Finnish and Russian populations does not explain differences in allergy phenotype

To explore the role of genetic variation in explaining differences in allergy phenotype between the Finnish and Russian groups, we performed a genome-wide association analysis (Figures S1 and S2 and Table S2 and in the Appendix S1).

An unsupervised approach was used to evaluate whether the sample dataset could be stratified into two populations. Both PLINK and fast-Struc analyses suggested, however, the absence of genetically distinct group based on genetic data. Next, we analysed difference in allelic frequencies between Russian and Finnish populations using a supervised approach. We found several single-nucleotide polymorphisms (SNPs) that were significantly different between the two cohorts. However, these differences explained only 1.19% of the genetic variance and were spread over several thousand loci. Moreover, no significant genetic differences associated with allergy phenotype could be observed.

In order to collectively evaluate biological functions of the observed genetic variation, we identified SNPs-associated genes (*n* = 31 genes) that were significantly different between the Finnish and Russian subjects and used them for GO enrichment analysis. No significant enrichment of any biological processes, cellular components, or molecular functions was found.

3.2 | High-total IgE levels are associated with enhanced anti-viral response in Finnish but not in Russian subjects

Sensitization to common allergens was much more common in the Finnish subjects compared with Russians, for example to birch

pollen 35% vs 7%, to cat 26% vs 7%, and peanut 12% vs 0% (IgE level ≥ 0.35 kU_A/l) (Table S1 in the Appendix S1).

Genome-wide transcriptomic analysis was done to investigate differences in the gene expression between sensitized and non-sensitized subjects. Blood leucocytes (PBMC) from pollen sensitized and non-sensitized Finnish and Russian subjects were investigated, but no differentially expressed genes (DEGs) were found either in Finnish or in Russian population (data not shown). However, when the subjects were divided into low-serum-IgE (low-sIgE; total serum IgE <110 kU_A/L) and high-serum-IgE (high-sIgE; total serum IgE >110 kU_A/L) groups differential analysis revealed 51 DEGs (adjusted *P*-value <.05 and log2 fold change $-0.33 < \text{or} > 0.33$) in FIN high-sIgE (*n* = 36)/low-sIgE (*n* = 33) contrast (Table S3 in the Appendix S1). Forty-five of the DEGs were up-regulated and 6 were down-regulated. Only 6 DEG were identified between RUS high-sIgE (*n* = 26) and low-sIgE (*n* = 49) individuals. DEGs from the Finnish individuals clustered quite well according to low-sIgE and high-sIgE groups (Figure 1A).

For functional insight, we performed Gene Ontology (GO) enrichment analysis using PANTHER overrepresentation test. Significant enrichment of anti-viral and interferon signalling-related pathways was seen in both GO biological processes and Reactome pathways (Figure 1B). Moreover, several major interferon-induced cytokines (eg IFIT1, IFIT3, IFI27) were up-regulated in FIN high-sIgE group but not in RUS high-sIgE group (Figure 1C). The analysis did not identify significantly enriched functions in 6 DEGs derived from the RUS high-sIgE/low-sIgE comparison.

3.3 | Skin microbiota and transcriptomes are highly contrasting between subjects from the Finnish and Russian Karelia

We continued to investigate transcriptomic differences underlying allergy disparity between the countries at the population level. Genome-wide transcriptomic studies of mononuclear blood cells revealed 261 differentially expressed genes (DEGs, adjusted *P*-value <.05 and log2 fold change $-0.58 < \text{or} > 0.58$) between Finnish and Russian populations, which tended to cluster the countries fairly well (Figure 2A). Interestingly, a large part of the up-regulated genes (*n* = 25) in Russian subjects belong to a group of long non-coding RNA (lncRNA), while one third of the down-regulated genes were immune-related (Table S4 in the Appendix S1).

The transcriptomic results are paralleled by differences in skin microbiota between Finns and Russians (Figure 2B,C). Nationalities are clearly clustered along both microbial composition and gene expression (Figure 2B). Along the microbial dimension, 99% of variation could be accounted to nationality, while along the gene dimension 92% of variation could be explained by nationality (using a linear model). Thus, it is not surprising that the separation of nationalities due to the co-dispersion along these two axes is highly significant (MANOVA: $P < 2e-16$). While there was a significant overall Mantel correlation between gene expression and microbial composition when (Table S2 in the Appendix S1), this could not be observed within nationalities, likely due to limited sample size.

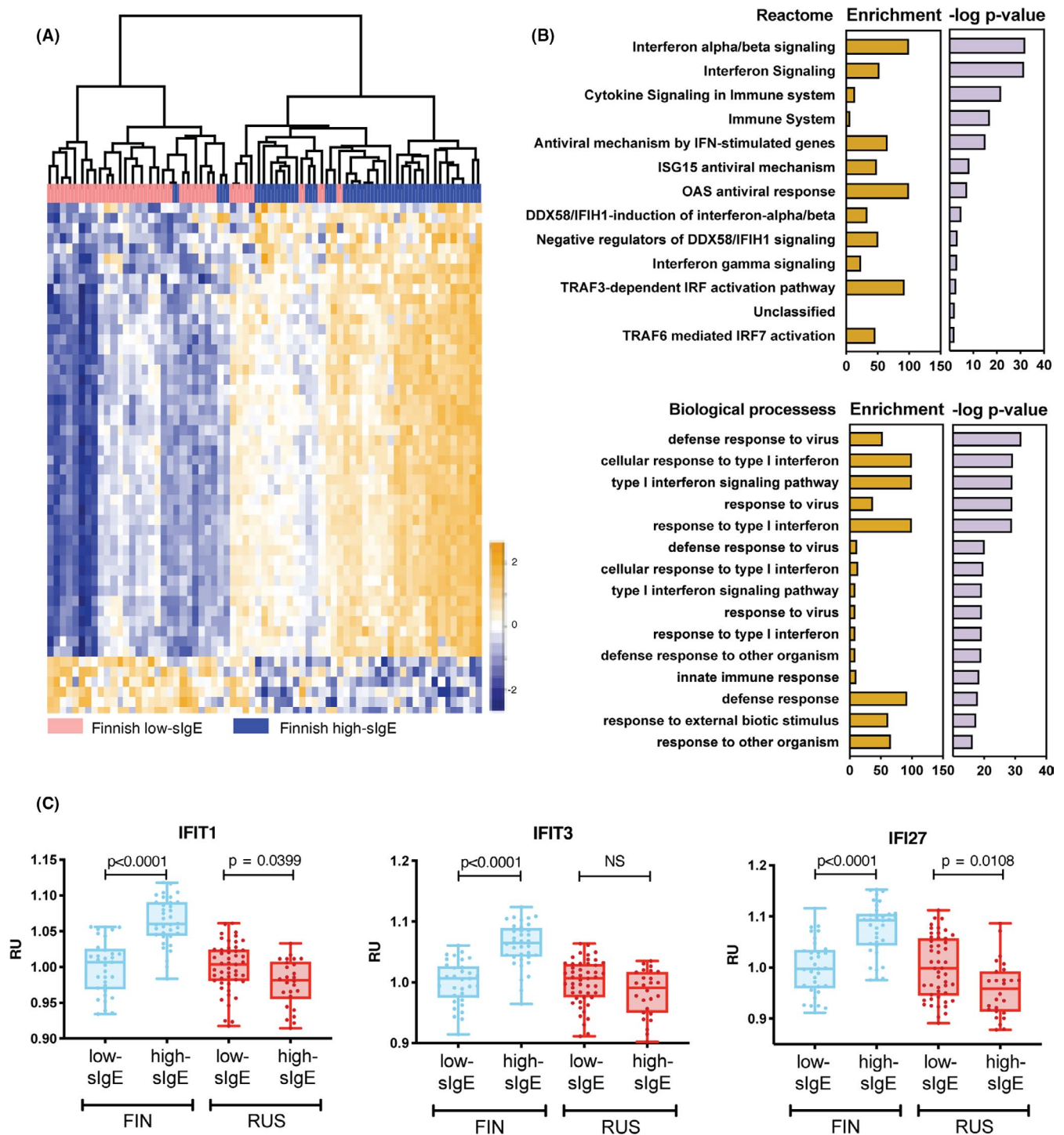


FIGURE 1 A, Hierarchical clustering of differentially expressed genes between low-slgE ($n = 33$) and high-slgE ($n = 36$) Finnish individuals. The heat map gives normalized (z-scored) expression levels of 51 genes. B, Enrichment of Reactome pathways and GO biological processes of the differentially expressed genes derived from the Finnish high/low-slgE contrast. C, Expression of IFIT1, IFIT3, and IFI27 interferon-inducible genes in Finnish and Russian low-slgE and high-slgE groups

The Ingenuity Pathway Analysis of DEGs revealed suppression of innate immune functions in the Russian samples compared with the Finnish samples. Especially, the recognition of bacterial and viral components (*TREM1*, *PRR* in recognition), Toll-like receptor activation, and inflammatory responses (*IL-17F*, *inflammasome*, *IL-6*, *IL-8*) were decreased in Russian samples (Figure 3A). Moreover,

Cellular movement-associated molecular functions (Figure 3B) were suppressed in Russian samples. As an example of immune molecules belonging to canonical pathway "Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses" expression of major proinflammatory cytokines, *IL-1-beta* and *IL-6*, as well as key toll-like receptors, *TLR2* and *TLR4*, are shown in Figure 3C.

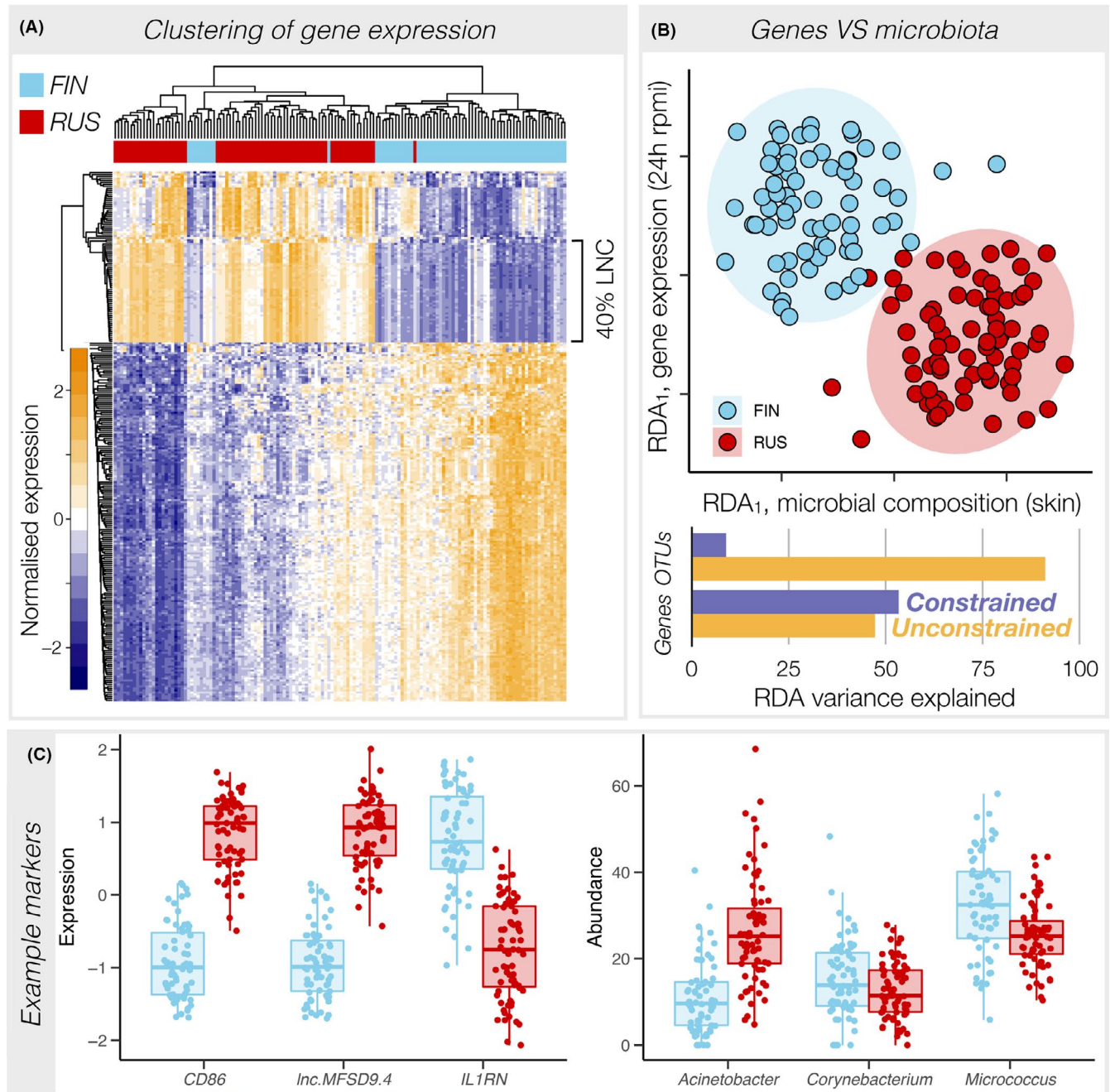


FIGURE 2 A, Clustering of differentially expressed genes (24 h rpm) in PBMC between populations (FIN vs RUS). The heat map gives normalized (z-scored) expression levels of 267 genes in 69 Finnish and 73 Russian subjects. Dendrograms represent hierarchical partitioning of samples and genes based on Ward's method. B, Reduced-dimension representation (1st RDA axis modelling between-population difference, while correcting for age and gender) of skin microbiota (8.8% of variation explained; *Constrained*) and PBMC gene expression (55% of variation explained; *Unconstrained*) plotted against each other. Below, the amount of constrained and unconstrained variation for both RDA models. C, Examples of gene expression patterns differing between populations (left) and of microbial genera showing both similar and differing abundances between populations (right)

3.4 | Gene-microbe network is much larger in the Russian vs Finnish subjects

Using network analysis, we tested whether gene expression patterns differ between countries. Since gene and OTU data inherently follow different distributions, the analysis was based on discretized features. The overall topology of the co-expression networks was

similar in both populations (Figure 4A), with the network of the Finnish subjects more strongly connected, measured by graph closeness, than that of the Russian subjects (Figure 4B).

However, the set of connections between genes and skin OTUs was significantly richer among Russian ($n_+ = 25$, $n_- = 34$) than Finnish subjects ($n_+ = 9$, $n_- = 11$), with at least twice as many OTUs relative to total vertex count (number of nodes) and double the amount of

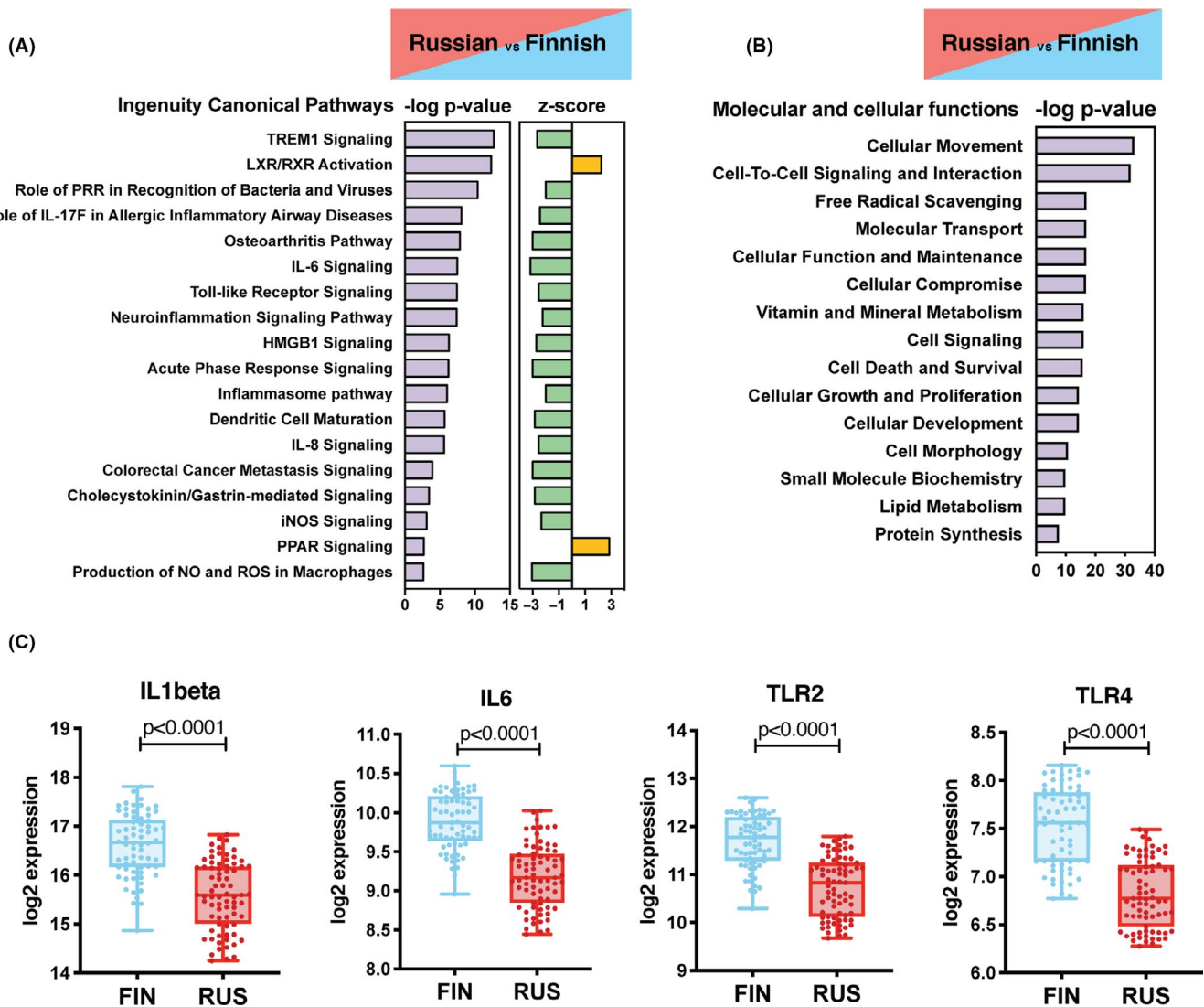


FIGURE 3 A, Ingenuity pathway analysis (IPA) of differentially expressed genes in Russian/Finnish contrast. B, Top IPA molecular and cellular functions in Russian/Finnish contrast. C, Expression of major proinflammatory cytokines, IL-1-beta and IL-6, and toll-like receptors, TLR2 and TLR4. Example, belonging to canonical pathway "Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses" in Finnish and Russian subjects

gene-OTU edges (Figure 4B). Focusing only on subgraphs containing OTU-gene edges revealed differences in network size and complexity between populations (Figure 4C) indicating a more diverse association between microbes and gene expression in the Russian vs Finnish subjects. In the Russian subgraph, top functions associated with neutrophil accumulation (B-H adjusted $P = 1.9 \times 10^{-3}$) and leucocyte influx (B-H adjusted $P = 1.9 \times 10^{-3}$) were significantly enriched (Figure 4D), and were inhibited by genes associated with microbes in IPA analysis.

3.5 | High *Acinetobacter* abundance correlates with suppression of innate immune functions in the Russian subjects

Given the important role of *Acinetobacter* in previous reports,^{4,13,18} we stratified and analysed subjects according to the abundance of

Acinetobacter on their skin (including 1st and 4th quartiles in the analysis).

Transcriptomics analysis revealed 76 DEGs in *Acinetobacter* high ($n = 19$) compared with *Acinetobacter* low ($n = 18$) Russian individuals (Table S5 in the Appendix S1), which clustered the two groups fairly well (Figure S4 in the Appendix S1). Significant correlation between chemokine expression (eg CCL2, CCL3, CXCL3, CXCL5) and *Acinetobacter* abundances is shown in Russian *Acinetobacter* high and *Acinetobacter* low individuals (Figure S5 in the Appendix S1).

Ingenuity pathway analyses identified the enrichment of molecular functions related to cell movement/migration, activation of cell death, and intestinal inflammation (Figure 5A). The same functions that were suppressed or activated in blood mononuclear cells, when comparing the Finnish and Russian samples, behaved similarly when comparing Russian *Acinetobacter* high and *Acinetobacter* low samples (Figure 5A). In line with this, the pattern of expression of chemokines, CCL2, CCL3,

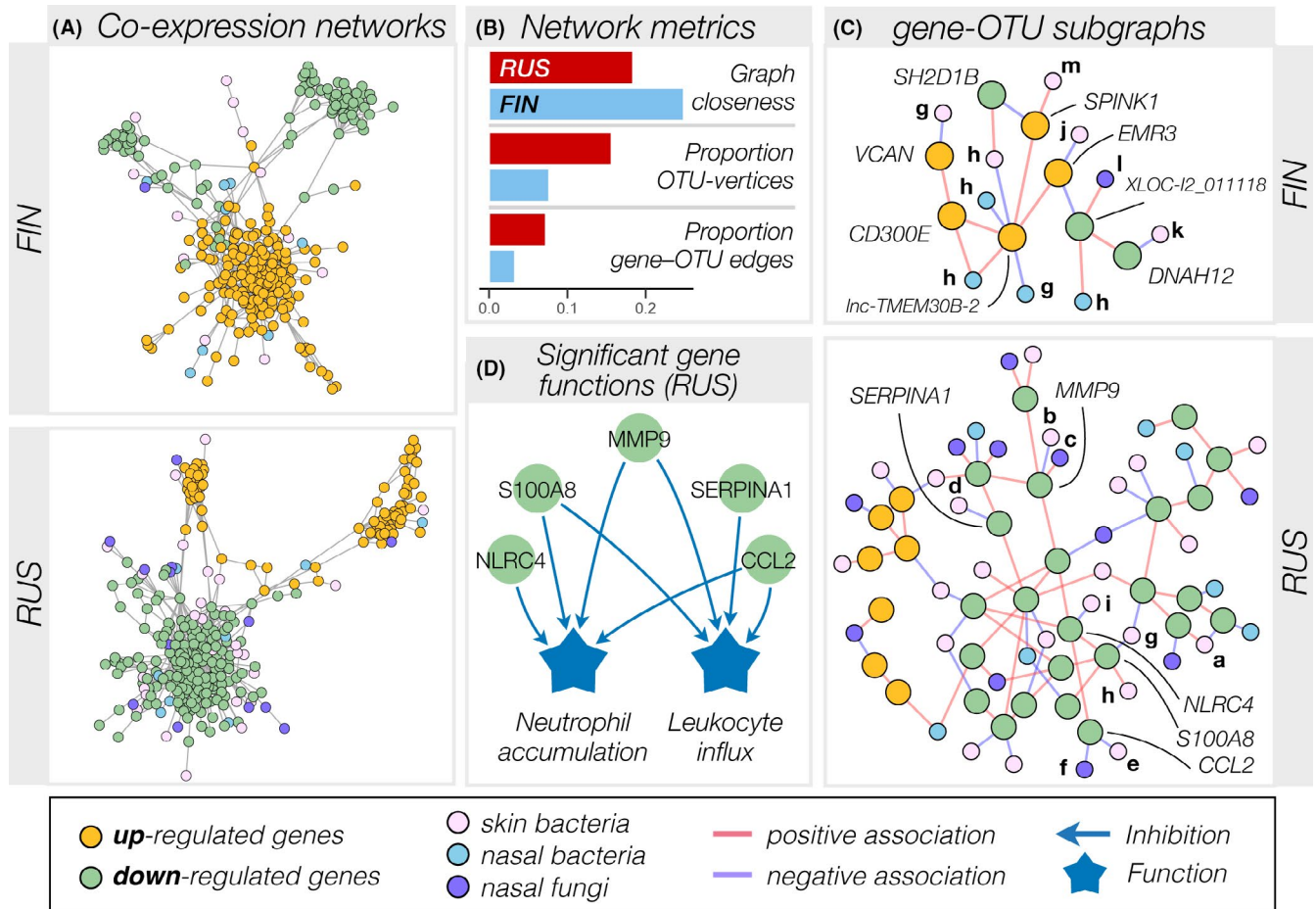


FIGURE 4 A, Differentially expressed genes in Finnish (Finnish) and Russian (Russian) Karelia populations display superficially similar co-expression networks with skin or nasal bacteria or nasal fungi (OTU) (networks were generated with the bc3net algorithm). B, The Finnish network is more tightly interconnected than the Russian network (measured as graph closeness), but the Russian network has proportionally more OTUs (vertices) and connections (edges) between genes and OTUs than the Finnish network. C, Largest gene-OTU subnetworks: the Finnish subnetwork is smaller than the Russian one (vertex count 18 vs 71). The letters indicate microbial OTUs: a = *Acinetobacter*, b = *Aerococcus*, c = *Aspergillus*, d = *Cryseobacterium*, e = *Streptococcus*, f = *Thedgonia*, g = *Staphylococcus*, h = *Corynebacterium_1*, i = *Kocuria*, j = *Micrococcus*, k = *Dietzia*, l = *Malesseria*, and m = unclassified *Proteobacterium*. (d) In the Russian subnetwork, the most significantly enriched functional pathways are associated with neutrophil accumulation and leucocyte influx, both being inhibited by five down-regulated genes

and CXCL5, is highly similar in FIN/RUS contrast compared to RUS *Acinetobacter* high/RUS *Acinetobacter* low contrast (Figure 5B). Finally, stimulation of PBMCs with *A. lwoffii* extract elicited expression of major proinflammatory cytokines, IL6 and IL1beta, only in the Finnish but not in the Russian samples (Figure 5C), in line with our observations.

4 | DISCUSSION

In this study, we have demonstrated that geographically adjacent, but socio-economically distinct populations in Finnish and Russian Karelia differ in their immune status, potentially due to differing microbial exposures affecting immune regulation.^{3,19,20} Our study is observational by design and does not allow assessment of causal relationships, which need further experimental studies. Nevertheless, several partly independent lines of evidence point

to the same direction. The more diverse microbial exposure on the Russian side promotes immune tolerance, which seems to associate with the low rate of clinical allergy.¹ The low number of allergic subjects on the Russian side prevented further country-specific comparisons.

Firstly, we evaluated the possible contribution of genetic variation between the Finnish and Russian populations. However, no stratification into two populations could be found using unsupervised genetic analysis. Furthermore, no association between allergy phenotype and allelic variation could be found. Although significantly different SNPs between the two populations were identified, these differences explained only about 1% of the genetic variation and were distributed over several thousand loci. Moreover, no enrichment of any biological functions was found in SNP-associated genes and none of these genes were identified in our transcriptomic analysis. It is likely that the observed genetic differences are index of ongoing genetic differentiation

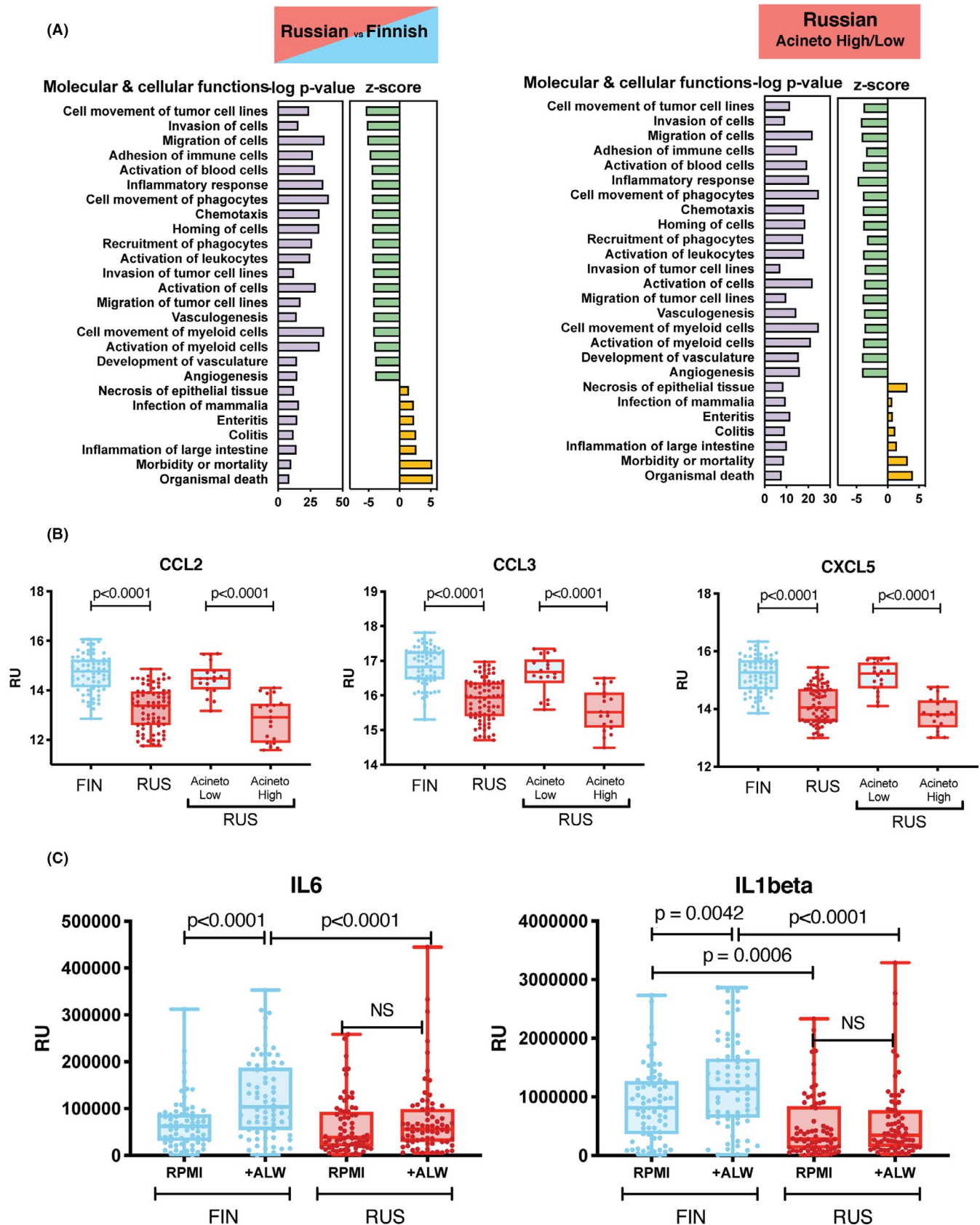


FIGURE 5 A, Comparison of IPA molecular functions in the Russian/Finnish group and in the Russian *Acinetobacter* high/low group. B, Expression of IFIT1, IFIT3, and IFI27 interferon-inducible genes in the Russian/Finnish group and in the Russian *Acinetobacter* high/low group (C) Expression of proinflammatory cytokines IL6 and IL1beta in response to stimulation with *A. lwoffii*

between the populations and has little, if anything, to do with the microbe-host interaction mediated immune regulation observed in the present study. Nevertheless, conclusions drawn from the GWAS study should be interpreted with caution since the power to detect significant associations is low due to the small sample size.

Secondly, we investigated transcriptomics differences in the blood leucocytes related to allergic sensitization. No significant differences between pollen sensitized and non-sensitized Finnish or Russian subjects were found, which is likely due to the relatively small sample size and a modest variation in gene expression in the steady-state condition. However, transcriptomic profile of Finnish subjects with high-serum-IgE levels exhibited strong enrichment of anti-viral and interferon signalling-related pathways. Such enhanced anti-viral type profile was not seen in Russian high-sIgE individuals suggesting better balanced immune regulation in Russian side compared with Finnish side. We have previously reported in patients with seasonal allergic rhinitis (SAR) that an exaggerated anti-viral response is triggered in nasal epithelia upon seasonal exposure to environmental stimuli.²¹ It is of interest that 6 differentially expressed genes which were strongly related to interferon signalling (ie CMPK2, DDX58, IFIT1, IFIT2, IFIT3, MX1) were shared between the current blood transcriptomics study and our previous nasal epithelia proteomic study.²¹ We may speculate that over-activated anti-viral-type response in Finnish high-sIgE individuals during allergen exposure leads to abnormal production of interferon-inducible proteins finally leading to exaggerated inflammatory reactions that contribute to the disease phenotype.

Thirdly, we examined the gene expression profiles at the population level to elucidate molecular explanation underlying variation of immune regulation between the Finnish and Russian populations. Transcriptomic analysis revealed significantly lower levels of inflammatory function on the Russian side compared with Finnish side, including innate immune signalling and cell movement. Interestingly, a large part of the transcripts that were up-regulated in the unstimulated blood leucocytes in the Russian subjects were identified as long non-coding RNAs (lncRNAs). This new class of regulatory RNAs plays a pivotal role in the regulation of gene expression, and is involved in immune cell development and function.²² Moreover, lncRNAs may play a role in controlling host immune responses during microbial infection.²³ Indeed, our network analysis, revealing significantly richer gene-microbial interaction patterns in the Russian population, indicates a link between transcriptional and microbial differences between the two populations.

Finally, we observed a pattern of suppressed innate immune responses in individuals exhibiting high abundance of *Acinetobacter*, with high immunoregulatory potential,^{13,18} compared with individuals revealing low levels of *Acinetobacter* on their skin and nasal epithelium. This was observed both in unstimulated and *Acinetobacter*-stimulated cell cultures. Importantly, the transcriptomic patterns between high and low *Acinetobacter* individuals from Russian Karelia recapitulated the between-population (Finnish vs Russian) differences in the regulatory pathways. This suggests that the between-population differences are deeply rooted in the dynamics of host-microbial interaction.

Our results are not due to technical artefacts. Firstly, only about 3% of the gene transcripts examined were differentially expressed

between the populations. One third (34%) of the down-regulated genes were associated with immune function. Secondly, gene-gene co-expression patterns were highly similar between the populations, while the major difference was due to the differential gene-microbe co-expression. The major constituents of microbial communities were similar between the populations, but the microbiota of Russian subjects were both more diverse and richer in microbes of environmental origin.²⁴ Finally, when comparing subjects with either high or low abundance of *Acinetobacter* on their skin revealed that the country difference in the activity of regulatory pathways was repeated among the Russian subjects. Taking together, the between-population differences are not systematic; only a few markers differ. Still, especially the differences in gene functions are systematic, and they all tend to be associated with immune function.

We propose that lifelong exposure to environmental microbiota—more intense and richer in diversity on the Russian side^{3,20}—influences individual immune function and results in a better balanced immune system than that observed in the Finnish Karelian population. This conclusion is in line with both experimental and empirical studies and underlines the instrumental role of microbiota in the development of balanced immune tolerance.^{13,25–27} While in agreement with the results of Stein et al,¹¹ comparing populations performing either modern or traditional farming, our results generalize the role of lifestyle in microbial exposure to a broader context of environmental influences.

The Finnish and Russian Karelia cohorts provide an opportunity to explore the role of the human microbiota in the development of allergy and in the overall immune homeostasis. Biologically inappropriate inflammatory responses to pollen proteins are manifold in the Finnish population, and this difference has mostly developed after the Second World War.²⁸ Western lifestyle has also been connected to many other chronic inflammatory conditions.²⁹ Revealing the reasons for allergy disparities may pave the way not only to understand allergies but also several other harmful inflammatory responses.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

LR contributed to study design, data analysis, data interpretation, figures, and writing. NF contributed to data generation, data interpretation, and writing. TL contributed to cohort design, data collection, and manuscript commenting. PA contributed to data generation, data interpretation, and manuscript commenting. VF contributed to data analysis

and manuscript commenting. GS performed genome-wide association analysis and manuscript commenting. PJ contributed to cohort design, data collection, and manuscript commenting. PK contributed to data generation and manuscript commenting. JV contributed to data generation and manuscript commenting. AK contributed to data analysis and manuscript commenting. OM contributed to data collection and manuscript commenting. MJM contributed to data interpretation, figure commenting, and manuscript commenting. SL contributed to data collection and manuscript commenting. JN contributed to data interpretation and manuscript commenting. NO contributed to data interpretation and manuscript commenting. LP contributed to cohort data collection and manuscript commenting. EV contributed to cohort design, data interpretation, and manuscript commenting. LVH contributed to data interpretation and manuscript commenting. DG contributed to data generation, data analysis, data interpretation, and manuscript commenting. TH contributed to study design, data interpretation, figure commenting, and writing. HA contributed to study design, data collection, data analysis, data interpretation, figures, and writing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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